Enhanced Macrophage Cholesterol Efflux Capacity in Middle-Aged Adults with Childhood-Onset Type 1 Diabetes: A Cross-Sectional Analysis

by

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Abstract

Background

Individuals with type 1 diabetes (T1D) exhibit higher rates of coronary artery disease (CAD) compared with the general population, despite higher concentrations of the cardioprotective HDL-C, thus we hypothesized that HDL may not be a good indicator of HDL function. This study assessed differences in novel markers of HDL particle concentration (HDL-P) and macrophage cholesterol efflux capacity (CEC) between middle-aged adults with childhood-onset T1D and controls with normal glucose tolerance (NGT).

Methods

HDL-P and CEC were determined among 187 individuals with T1D (mean age 51 years, T1D duration 43 years, 52% women) and 200 controls of similar demographic distribution. As a larger number of those with T1D (n=44) had CAD compared to controls (n=4), analyses were restricted to individuals free of CAD to assure more comparable groups. HDL-P and macrophage CEC were quantified by calibrated ion mobility analysis and a validated cell-based assay, respectively. Descriptive analyses were conducted to assess differences by sex and T1D status, as well as the relationships of the novel HDL markers. Separate multivariable linear regression models were constructed for each novel HDL marker to evaluate whether T1D status was an independent correlate. The presence of effect modification by sex was also assessed.

Results

Individuals with T1D and women (regardless T1D status) exhibited favorable traditional lipid profiles. Significant sex differences (regardless T1D status) were also detected in total HDL-P (higher in women) and the four major HDL subpopulations – extra-small (higher in men), small (higher in men), medium (higher in women), and large (higher in women), although concentrations were similar by T1D status. Total macrophage CEC was higher in those with T1D (all $p<0.01$). T1D was associated with 1.06 μmol/L lower M-HDL-P concentrations but 0.39 μmol/L higher L-HDL-P concentrations and 0.26% higher total macrophage CEC compared to NGT ($p=<0.01$), after sex and HDL-C adjustment.

Conclusion

In this case-control study of middle-aged adults free of CAD, individuals with childhood-onset T1D had lower concentrations of M-HDL-P but higher levels of L-HDL-P and total macrophage CEC. Further research is needed to contextualize these findings and expand upon the public health implications of HDL dysfunction.

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Preface

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I would like to thank my research mentor and essay advisor Dr. Tina Costacou, for her patient and thoughtful guidance throughout my time at Pitt. I would also like to thank my academic advisor Dr. Nancy Glynn, whose dedication to the success of her students is unmatched. Both Dr. Costacou and Dr. Glynn have contributed greatly to my education and made it possible for me to be successful at Pitt, and for that I am eternally grateful. I would also like to thank Dr. Ada Youk and Dr. Thomas Songer for their keen eyes and thoughtful comments throughout the drafting process of this essay. Finally, I am grateful for the close friendships forged in spite of a global pandemic, and for the kind hearts and open minds I've encountered along the way. Our experience has been unique, and our bonds are that much stronger for it.

1.0 Introduction

1.1 Type 1 Diabetes Pathophysiology

Type 1 Diabetes is an autoimmune disease characterized by the destruction of insulin-producing pancreatic beta cells. The hormone insulin acts as a facilitator by promoting glucose uptake in cells and regulating carbohydrate, lipid, and protein metabolism.[1] In the presence of deficient insulin concentrations, individuals will begin to experience symptoms of type 1 diabetes, including polyuria, polydipsia, and polyphagia.[2] Insulin deficiency in type 1 diabetes prevents the body from regulating blood glucose, leading to increased concentrations of glucose in the blood, also known as hyperglycemia.[3]

The exact process leading to the destruction of beta cells is largely viewed as an autoimmune process, with multiple facets involved in its initiation, including an environmental trigger in genetically susceptible individuals. However, the exact mechanism of this autoimmune initiation is not clearly understood.[4,5] The pathophysiology of type 1 diabetes can be categorized into three distinct stages: stage 1 involves the persistent presence of multiple β-cell autoantibodies with normoglycemia, or normal concentrations of glucose in the blood. stage 2 is further defined by the development of dysglycemia, or the dysregulation of glucose in the blood, and stage 3 is characterized by the onset of clinical symptoms,[6] such as polyuria, polydipsia or polyphagia.

The diagnosis of type 1 diabetes may be made with either: a) a fasting plasma glucose $\geq$126 mg/dL (7.0 mmol/L), b) 2-hour plasma glucose $\geq$200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test, c) HbA1c $\geq$6.5% (48 mmol/mol), or d) a random plasma glucose $\geq$200 mg/dL (11.1

mmol/L) in the presence of symptoms of hyperglycemia or hyperglycemic crisis. In the absence of symptoms, diagnosis requires two abnormal test results.[6]

The onset of type 1 diabetes is typically protracted, as it takes time for the body to stop producing insulin. Treatment is most notably distinguished by the immediate and lifelong need for exogenous insulin. The period following diagnosis, during which the ß cell count dwindles, is known as the "honeymoon phase" and varies from person to person, lasting weeks, months, or even years. Type 1 diabetes typically stabilizes after the honeymoon phase; care subsequently becomes more predictable.[7] Nonetheless, prolonged hyperglycemia has been associated with complications of the micro- and macrovascular system in type 1 diabetes. Long term complications span the macro- and microvascular spectrum, including but not limited to retinopathy, nephropathy, neuropathy, and coronary artery disease.[8]

1.2 Long Term Care

A diagnosis of diabetes was considered fatal until 100 years ago when the hormone insulin was discovered. Exogenous insulin use has varied in implementation since the discovery of insulin, and both the method of delivery and the type of insulin used has changed drastically. Today, a commonly used standard of care is a semi-closed loop system of insulin delivery via a combination of continuous glucose monitoring and insulin pump technology.[9] Insulin delivery affects the blood glucose level, and treatment has the goal of stabilizing blood glucose to the normal range of between 70 and 130 mg/dL. Achieving normal levels of blood glucose is complicated by factors affecting blood glucose levels such as diet and exercise, making diabetes care a dynamic process.

An important biomarker that is often used as a metric for overall glycemic control is hemoglobin A1c% (HbA1c%). HbA1c% is a measurement of glycated hemoglobin over a period of three months and is used to monitor glycemic control during this time period in people with diabetes.[10] Long term care on the individual level is thus characterized by a constant balancing act of treating hypo/hyperglycemia to better achieve glycemic control.

1.3 Epidemiology

1.3.1 Incidence and prevalence

Type 1 diabetes incidence and prevalence have been on the rise globally for many years. Incidence has been identified to rise by 2-3% per year, with an annual incidence in the US of about 22.9 cases per 100,000 as of 2015.[5] While this increase varies by geographic location and season, there is little dispute that type 1 diabetes is a public health concern of global scope. Traditionally, type 1 diabetes has been thought of as a disease most often diagnosed in children 10-14 years of age and remains one of the most frequent chronic diseases diagnosed in children in the western world, although recent studies suggest it occurs equally across all age groups.[11,12] These numbers are reflected in the prevalence of type 1 diabetes, with 3.4/1,000 individuals in the United States being diagnosed before they are 40 years of age.[13]

The incidence of type 1 diabetes has been reported to be slightly higher in boys compared to girls in the U.S.,[14] although it has been shown that sex interacts with geography, such that as incidence increases by location, the sex ratio of diagnosis moves from a female excess to a male excess.[15] The incidence of type 1 diabetes is highest in individuals of northern European descent,

with an incident rate of 23.6/100,000. However, incidence has been rising in minority communities as well.[16] Indeed, type 1 diabetes incidence is rising fastest in Hispanic, Asian/Pacific islanders and Black individuals, with an annual incidence increase of 4.2%, 3.7% and 2.2% respectively, compared to an annual increase in non-Hispanic whites of 1.2%.[14]

1.3.2 Risk Factors

A variety of risk factors contribute to the development of type 1 diabetes. Major risk factors include genetic susceptibility and environmental factors such as exposure to viral infections.[17] Age has historically been an important factor for describing type 1 diabetes diagnosis, with the disease traditionally developing in childhood. It is now understood that type 1 diabetes can also develop in adults. In addition, a meta-analysis of 5 cohort and 25 case-control studies suggested a 5% increase in the odds of type 1 diabetes per 5-year increase in maternal age at birth.[18] Although weak, these data, along with increases in the age women give birth in modern societies, may help explain some of the observed increase in the incidence of type 1 diabetes over time.

Genetic susceptibility is a principal risk factor in the processes leading to the development of type 1 diabetes. The cumulative incidence of type 1 diabetes among monozygotic twins is 65%.[19] There are two HLA haplotypes that are largely implicated in the risk of developing type 1 diabetes. The HLA-DR3-DQ2 and the HLA-DR4-DQ8 haplotypes are present either alone or in combination in almost 90% of children diagnosed with type 1 diabetes.[17,20] HDL-DR15-DQ6, on the other hand, is associated with a decreased risk of developing diabetes, although the mechanisms for this association are not well established. Since the widespread use of genomic sequencing, over 60 additional genetic loci have been indicated as possible factors for the development of disease.[21]

Genes associated with type 1 diagnosis are typically thought to act in concert with some environmental trigger, including modifiable factors such as exposure to viral infections and lifestyle factors such as diet. Enteroviruses are some of the earliest environmental triggers to be studied, and have been implicated in prospective cohort studies as major triggers in islet autoimmunity.[22,23] This has been shown on a molecular level as well, providing strong evidence of increased risk.[24] In addition to viral exposure of the child, maternal exposure to enteroviral infections during pregnancy have also been associated with an increased risk of type 1 diabetes in the offspring.[25,26]

Data also exist suggesting that dietary factors are important contributors to the pathogenesis of type 1 diabetes. The role of breastfeeding and infant formula / cow's milk during the first few months after birth has received considerable attention, although overall findings remain inconclusive.[27–30] Cow's milk has been suggested to play a role in the development of type 1 diabetes in genetically susceptible individuals.[31] However, studies have shown mixed results, and additional research studies are needed to further assess this relationship.[32] Several studies have also suggested that Vitamin D concentrations during pregnancy may play a protective role against the development of beta cell autoimmunity and type 1 diabetes in the offspring, although not all studies concur.[33–38] The evidence available to date therefore suggest that the environmental trigger component is a complex aspect of diabetes development and requires further examination.

1.4 Complications Associated with Type 1 Diabetes

While diabetes is no longer considered a terminal disease, this disorder often entails meaningful future health conditions affecting quality of life and life expectancy.[39,40] Despite

persistent advances in care, this chronic condition has high costs both financially and mentally associated with daily upkeep, including prevention of severe hypoglycemia as well as more long-term micro and macrovascular outcomes. The societal financial burden of type 1 diabetes has been estimated to be $602 billion and growing, and the per-person cost difference attributable to the disease between people with normal glucose tolerance and type 1 diabetes has been estimated to be $800 per month.[41,42] In addition to both societal and per-person financial cost, there are burdens on mental health; depression rates in people with type 1 diabetes are more than three times that of the general public [43] and are associated with complications.[44,45]

1.4.1 Acute Complications

Acute complications of type 1 diabetes include the threat of diabetic ketoacidosis (DKA), a life-threatening complication in which the body does not have enough insulin to use the glucose in the blood stream and begins to produce dangerously high levels of ketones, as well as severe hypoglycemic events. DKA commonly presents at the time of the diagnosis of type 1 diabetes and is caused by very low concentrations of insulin due to beta cell failure, leading to impaired utilization of glucose, which in turns leads to hyperglycemia and increased production of ketones. Approximately a third of individuals with type 1 diabetes present with DKA at diagnosis in the U.S.; risk factors for DKA comprise younger age, lower socioeconomic status and minority race/ethnicity.[46]

Hypoglycemia is defined as abnormally low concentrations of blood glucose which, depending on their severity, may lead to mild cognitive impairment, coma, seizure or sudden death. Symptoms of hypoglycemia are often characterized by tremors, sweating, hunger and anxiety.[47] The incidence of severe hypoglycemia in children has been estimated to be 19 per 100 person years

in 0-19 year olds [48] and 8 per 100 person years among 7-16 year olds. It has been estimated that 4% to 10% of type 1 diabetic deaths are associated with severe hypoglycemia.[49] Intensive glycemic control is a strong risk factor for developing frequent hypoglycemic events, as is age and longer duration of diabetes.[50]

1.4.2 Microvascular Complications

Several diabetes-related comorbidities are microvascular in nature. Neuropathy is a form of nerve damage that is attributable to the peripheral impaired blood supply to the small blood vessels, most notably in the feet.[51] This painful disease has been estimated to affect over half of all people living with diabetes.[52] In the Pittsburgh EDC study of childhood-onset type 1 diabetes, the prevalence of diabetic neuropathy was 34%.[52,53]

Diabetic retinopathy affects the majority of those with 20 years or more duration of type 1 diabetes, and is the leading cause of blindness in adults.[54] Duration of diabetes is highly correlated to the development of proliferative retinopathy; rates of retinopathy increase with longer diabetes duration. The incidence rate of proliferative retinopathy during the DCCT/EDIC study was 50%.[55]

Nephropathy and renal complications are a leading cause of excess mortality in persons with type 1 diabetes.[56] Historically, sex differences in incidence of macroalbuminuria and end-stage renal disease were present, with more men developing disease, but within subsequent generations this difference has faded.[57] Duration of type 1 diabetes has been the strongest predictor of nephropathy, and affects more than a quarter of the population by 40 years of duration.[58] Interestingly, renal disease has also been linked to increased risk of macrovascular complications such as coronary artery disease, further complicating the relationship of type 1 diabetes to vascular outcomes.[59]

1.5 Macrovascular Complications – Coronary Artery Disease

Coronary artery disease (CAD) is a cardiovascular disease characterized by angina pectoris (i.e., chest pain), arrhythmias (i.e., abnormal heartbeat), cardiomyopathy and heart failure. CAD is the leading cause of death in people with type 1 diabetes in most developed countries.[60,61] The pathogenesis of CAD involves inflammation of the arterial walls via the sticking of blood leukocytes to the surface of the wall, caused by a variety of pre-existing factors in patients.[62] This leads to calcification and hardening of the artery, buildup of obstructive plaque, ischemia, and eventually major cardiac events.

Approximately 2% of young adults with type 1 diabetes develop CAD, and almost 1% of those with diabetes for at least 20 years will experience a major cardiac event by age thirty.[63] Overall, individuals with type 1 diabetes are at a tenfold increased risk for CAD compared to those with normal glucose tolerance, although historically, glycemic levels have not shown a strong association with CAD incidence in prospective cohort studies.[64] However, recent results from the DCCT/EDIC clinical trial provided definitive results that intensive insulin therapy leads to significant reductions in CAD risk.[65]

Both nephropathy and neuropathy pose a threat as factors enhancing risk of CAD and major cardiac events in individuals with type 1 diabetes.[64] In addition to hyperglycemia and duration of diabetes, risk factors for cardiovascular disease in type 1 diabetes are similar to those in the general population and include smoking, dyslipidemia, hypertension, and inflammation.[63,66] An exception comprises male sex; in contrast to the general population, the incidence of CAD is similar in men and women with type 1 diabetes.

Historically, those with type 1 diabetes have had better risk factor profiles, including better overall lipid panels, with a higher concentration of the atheroprotective HDL-C, and lower rates

of obesity, compared with the general population, although more recently, obesity rates have been rising also in type 1 diabetes, such that they now correspond to rates observed in the general population.[63] The observation of similar or better standard risk factors in type 1 diabetes compared with the general population do not reflect the elevated risk for CAD in type 1 diabetes, presenting the question as to what the cause for such higher risk for CAD is. One potential explanation may relate to impairments in the function of the high-density lipoprotein (HDL) molecule.

1.5.1 HDL and Macrophage Cholesterol Efflux

HDL is well known to be cardioprotective and includes functions such as reduction of inflammation, reverse cholesterol transport, and the characteristic of being an antioxidant, leading to the promotion of overall coronary health. The function of HDL in reverse cholesterol transport is to prevent oversaturation of cholesterol in peripheral tissue by accepting and delivering it first to blood plasma and then the liver where it is directly excreted.[67] This is typically performed by macrophages in a process known as cholesterol efflux, wherein excess cholesterol is effluxed from the cells to extracellular receptors. HDL cholesterol efflux capacity (CEC), the ability of HDL macrophages to perform reverse cholesterol transport, has been shown to be inversely associated with incident cardiovascular events.[68,69]

Despite this known association, an increasing number of studies have provided evidence of a positive or null relationship between HDL cholesterol and coronary artery disease risk.[70] Moreover, although middle aged women transitioning through menopause are known to have a higher CAD risk, they can experience high levels of HDL-C.[71] These observations have led to the hypothesis that the cholesterol content of HDL may not accurately reflect its atheroprotective properties. These observations may also help explain the increased CAD risk in type 1 diabetes

despite highly elevated HDL-C concentrations. Indeed, results from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study suggested that a U-shaped association of risk exists in women with type 1 diabetes, such that the incidence of CAD increased both below HDL-C of 50 mg/dL and above 80 mg/dL.[72]

1.6 Gaps in Knowledge

New technology has been developed to assess the cholesterol efflux capacity of HDL, as well as concentration of HDL particles (extra small, small, medium, large). The functional capabilities of macrophage CEC have only recently become measurable, and further reporting on these novel HDL measurements is needed, especially where possible HDL dysfunction is hypothesized. Within the relational sphere of type 1 diabetes, HDL and CAD, there exists space for this hypothesized dysfunction of macrophage reverse cholesterol transport to be tested.

1.7 Public Health Significance

Type 1 diabetes is a chronic disease the incidence of which has been increasing worldwide. At present, primary prevention is not possible although type 1 diabetes management has improved greatly since the results of the DCCT/EDIC trial; there have been significant reductions in the complications of type 1 diabetes as well as a marked increase in life expectancy.[73] Nonetheless, type 1 diabetes continues to be associated with serious micro- and macro-vascular complications, which are largely responsible for the burden type 1 diabetes poses to both the individual patient

and society. In particular, the incidence of CAD is two to tenfold higher among young adults with type 1 diabetes compared with the general population, even though traditional risk factor levels, including the cardioprotective HDL-C, are similar or better among individuals with type 1 diabetes. Investigators have therefore raised the hypothesis that the cholesterol content of HDL may not be indicative of the atheroprotective properties of the HDL molecule and that novel measures of cholesterol efflux capacity and HDL particle concentration may best represent that function. Nonetheless, to date, data on differences in these novel metrics between individuals with type 1 diabetes and those with normal glucose tolerance are very scarce. This essay will therefore focus on the assessment of differences in HDL-mediated cholesterol efflux capacity and the concentrations of HDL particles in individuals diagnosed with childhood-onset type 1 diabetes and non-diabetic controls. This essay provides important data that may help explain the increased cardiovascular risk in type 1 diabetes and may subsequently also lead to novel therapies to reduce the excess cardiovascular risk imposed by type 1 diabetes.

2.0 Objectives

The objectives of this study were to assess differences in novel HDL function measurements (i.e., HDL cholesterol efflux capacity and the concentration of HDL particles) between individuals with type 1 diabetes participating in the Pittsburgh Epidemiology of Diabetes Complications study and individuals with normal glucose tolerance of similar age and sex distribution to those with type 1 diabetes. As it is well established that lipid concentrations differ by sex, stratified analyses by sex were also conducted. We hypothesized that individuals with type 1 diabetes have impaired cholesterol efflux capacity and HDL particle concentrations compared to those with normal glucose tolerance. We also hypothesized that these differences would be of greater magnitude among women, contributing further information to the debate over the factors involved in the increased risk in women with type 1 diabetes.

3.0 Methods

3.1 Study Population

This study is based on data from the RETRO-HDLc study, an ancillary of the Pittsburgh Epidemiology of Diabetes Complications (EDC) study. Study participants with type 1 diabetes were individuals with childhood-onset diabetes who were participating in the 25-year follow-up of the EDC study. Study participants without diabetes were of similar age, sex and racial distribution to the group of individuals with type 1 diabetes from the EDC study. The EDC study is a prospective cohort of individuals diagnosed with type 1 diabetes before age 17 who were diagnosed or seen within a year after initial diagnosis at Children's Hospital of Pittsburgh between 1950 and 1980. A first clinical assessment for the study occurred between 1986 – 1988. Mean age at first clinical assessment was 28 years and duration of type 1 diabetes was 19 years. Participants were followed subsequently with biennial surveys for 30 years and biennial clinical examinations for 10 years, as well as at the 18, 25, and 30-year follow up.

The RETRO-HDLc study has been described previously in detail.[74] Briefly, it was designed as an ancillary to the EDC study with the goal to determine whether HDL function is impaired in type 1 diabetes compared to individuals with normal glucose tolerance and whether novel HDL metrics (cholesterol efflux capacity, HDL particle size and concentration, as well as HDL proteomics) are associated with the risk of coronary artery disease in individuals with type 1 diabetes. Thus, the RETRO HDLc study was based on clinical and survey data as well as biologic specimen from individuals with childhood-onset diabetes who partook in the 25-year follow up clinical examination of the EDC (n=216) and controls recruited from registries within the

University of Pittsburgh as well as the University of Pittsburgh Medical Center (UPMC) between 2017 – 2018 (n=200). Control eligibility criteria included a similar age and sex distribution compared with EDC participants, absence of major morbidities and residence in the greater Pittsburgh area. 384 individuals expressed interest, of which 286 were screened and 262 were deemed eligible. 212 of the eligible control participants attended the clinic visit. During the visit, normal fasting glucose tolerance (<125 mg/dL) status was verified for 200 individuals for whom relevant survey and clinical data, blood and urine samples were collected, as in the EDC study.

3.2 Data Collection

Surveys regarding demographic and medical history information were collected from both controls and individuals with type 1 diabetes. Individuals with type 1 diabetes further completed surveys regarding diabetes self-care. The clinic visit included anthropometric evaluations and collection of fasting blood and urine samples. Weight and height were assessed and BMI calculated. Blood pressure was measured with a random zero sphygmomanometer after a 5-minute rest. Hypertension was defined as > 140/90 mmHg or use of antihypertensive medications. HbA1c was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY) and converted to DCCT-aligned HbA1c values using a regression equation derived from duplicate assays (DCCT HbA1c = (DCA HbA1c – 1.13)/0.81). Lipid panels were measured enzymatically (Cholestech LDX System, Alere, Hayward, CA, USA) and non-HDL cholesterol was calculated as total cholesterol minus HDLc. Serum and urinary albumin were assessed, in-house, by immunonephelometry[75]; white blood cell count (WBC) was obtained using a counter S-plus IV (Coulter Electronics, Hialeah, FL) and serum creatinine was assayed using an Ektachem 400 Analyzer (Eastman Kodak

Co, Rochester, NY). After closure of the in-house laboratory, assessment of serum and urinary albumin and creatinine as well as of WBC count for the RETRO-HDLc study was completed at Quest Diagnostics (Pittsburgh, PA). Glomerular filtration rate (eGFR) was estimated.[76]

HDL-P (the concentrations of HDL subspecies) was assessed using calibrated ion mobility analysis on a differential mobility analyzer (DMA, TSI Inc., MN). Four major HDL subpopulations (extra-small [XS-HDL-P], 7.6 nm; small [S-HDL], 8.3 nm; medium [M-HDL], 9.3 nm; and large [L-HDL],11.1 nm) were fitted to the DMA profiles by unsupervised, iterative curve fitting.[77] HDL cholesterol efflux capacity was quantified with serum HDL (plasma-derived serum depleted of apolipoprotein B lipoproteins) using cells labeled with [^{3}H]cholesterol. All assays were carried out under conditions where CEC was a linear function of incubation time and serum HDL concentration. Macrophage CEC was quantified with J774 cells stimulated with cAMP and was calculated as the percentage of total [^{3}H]cholesterol (medium plus cell-associated) released into the medium containing serum HDL minus that of cells incubated with medium alone. ABCA1 CEC was quantified using BHK (baby hamster kidney) cells with mifepristone-inducible human ABCA1. ABCA1 CEC was calculated as the percentage of total [H^3]cholesterol (medium plus cell-associated) released into the medium of BHK cells stimulated with mifepristone minus that of cells stimulated with medium alone. Analyses were normalized to a pool of control HDL that was included in each batch of assays. Laboratory personnel were blinded to the type 1 diabetes status of study participants.

3.3 Statistical Methods

A larger proportion of those with type 1 diabetes had CAD (22.5%) compared to controls (2%). We therefore restricted analysis to those free of CAD (n=245 individuals with type 1 diabetes and 196 controls) to ensure comparable populations. Analyses were further restricted to those with adequate sample for the novel HDL marker assessment. The final analytic sample size was 339 (143 individuals with type 1 diabetes and 196 controls without diabetes).

To evaluate the presence of an association between novel HDL metrics and participant characteristics, Pearson and Spearman correlations were generated, as appropriate, for normally and non-normally distributed variables, respectively. Descriptive analyses were then performed to assess differences in participant characteristics and novel HDL measures by sex and type 1 diabetes status. The Student's *t* test and the Wilcoxon's two-sample test were then performed to assess differences in normally and non-normally distributed continuous variables, respectively. The Chi-square test or Fischer's exact test, as appropriate, were employed for categorical variables. Separate multivariable linear regression models with backwards elimination were then constructed for each novel HDL marker as the dependent variable with type 1 diabetes status as the main independent variable, allowing for potential confounding factors. Confounding factors considered comprised sex, BMI, smoking status, hypertension, apolipoprotein A1, traditional lipid concentrations (HDL-C, non-HDL-C and triglycerides), ACR, eGFR, and WBC count. In addition, modification of the effect of type 1 diabetes on novel HDL metrics by sex was assessed. Analyses were performed using SAS version 9.4 (SAS institute, Cary, NC).

4.0 Results

Descriptive characteristics by sex and type 1 diabetes status are shown in Table 1. By design, the distribution of age and race/ethnicity was similar by sex and type 1 diabetes status. Overall, median age was 49.6 years and the vast majority (96%) were non-Hispanic white. Nonetheless, differences were observed in other participant characteristics by sex both within and between type 1 diabetes status groups, as described below.

Sex differences in controls: Among controls, women had a better risk factor profile compared with men, including a lower proportion of smokers and individuals with hypertension, lower WHR and higher HDL-C and apolipoprotein A1 concentrations; an exception was albumin to creatinine ratio (ACR), which was higher among women. Significant differences were also observed in novel HDL metrics by sex among controls, with women presenting with significantly higher cholesterol efflux capacity, as well as higher total, large and medium HDL-P but lower small and extra-small HDL-P concentrations.

Sex differences in type 1 diabetes: The sex differences observed among controls were largely mirrored within individuals with type 1 diabetes. Exceptions included the lack of a difference in the rates of smoking and hypertension as well as similar ACR and CEC by sex in type 1 diabetes. In addition, women with type 1 diabetes exhibited lower eGFR and reported a lower dose of insulin per bodyweight compared with men.

Overall differences by type 1 diabetes status: As expected, HbA1c was elevated among men and women with type 1 diabetes compared to controls. In addition, a greater proportion of men and women with type 1 diabetes had hypertension and used ACE/ARB and lipid medications. Study participants with type 1 diabetes also had higher concentrations of WBC count and ACR.

In contrast, lipid concentrations were more favorable in type 1 diabetes. Interestingly, despite similar concentrations of total HDL-P, men and women with type 1 diabetes also had significantly higher CEC (both total macrophage and ABCA-specific CEC). Among men, no differences were observed in the concentration of any of the HDL-P subspecies by type 1 diabetes status. However, among women, there was a shift toward lower medium HDL-P and higher large HDL-P in those with type 1 diabetes.

Table 2 describes correlations between the novel HDL markers and other characteristics of study participants stratified by type 1 diabetes status. Generally, the strength and direction of correlations were similar in both groups. The strength of correlations among novel HDL metrics and other characteristics was weak or moderate with the exception of correlations between HDL-C and total and large HDL-P, as well as between apolipoprotein A1 and total HDL-P, large HDL-P and total macrophage CEC, which were strong in both type 1 diabetes and controls.

Results of multivariable linear regression models are shown in Table 3. After multivariable adjustments, type 1 diabetes status was significantly associated with each of the HDL particle concentrations and both total macrophage and ABCA1-specific CEC measurements, however the direction of association varied. Having type 1 diabetes was associated with higher total macrophage and ABCA-1 specific CEC, higher concentrations of large HDL-P, yet lower concentrations of medium and total HDL-P after multivariable adjustment. An exception was extra-small and small HDL-P concentrations, for which no differences were observed by type 1 diabetes status. Adjusting for other risk factors and type 1 diabetes status, female study participants had lower extra-small and small HDL-P concentrations but higher medium HDL-P and marginally higher large HDL-P concentrations. Interestingly, although no differences were observed in total macrophage CEC by sex, women had significantly lower ABCA1-specific CEC compared with

men. Of traditional lipids, HDL-C negatively and non-HDL-C positively related to extra-small and small HDL-P. Furthermore, HDL-C positively related to large HDL-P. Traditional lipids (HDL-C, non-HDL-C and triglycerides) were also positively associated with CEC.

After multivariable adjustments, compared to controls with normal glucose tolerance, individuals with type 1 diabetes experienced a 0.26 ($\pm$0.02, p = <0.01) average unit increase of total macrophage efflux capacity. Models generally explained between 46% to 74% of the variability in HDL-P and CEC. This association was echoed in the other CEC measures as well; type 1 diabetes was generally associated with a higher CEC after taking the variables listed above into account. Results of multivariable linear regression also point to increased concentrations of large size HDL particles and decreased concentrations of medium size HDL particles in individuals with type 1 diabetes. There was not a significant interaction between sex and type 1 diabetes status, and the interaction term was subsequently dropped from analysis.

Table 1 Differences in participant characteristics by T1D status overall and within sex

	Controls			Type 1 Diabetes			Men (control vs. T1D)	Women (control vs. T1D)
Participant characteristics	**Men (n=97)**	**Women (n=99)**	**p-value**	**Male (n=64)**	**Female (n=79)**	**p-value**	**p-value**	**p-value**
ABCA-specific chol. efflux cap.	0.9(0.6,1.4)	1.1(0.6,1.4)	0.70	1.9(1.5,2.4)	1.9(1.1, 2.7)	0.31	<0.01	<0.01
Total macrophage efflux cap.	0.9(0.8,1.1)	1.0(0.9,1.2)	0.02	1.2(1.0,1.4)	1.3(1.1, 1.4)	0.20	<0.01	<0.01
XS HDL particle concentration	1.0(0.8,1.2)	0.8(0.6, 1.0)	<0.01	0.9(0.8,1.3)	0.8(0.7,1.0)	<0.01	0.97	0.64
Sm HDL particle concentration	3.8(3.0,5.0)	3.5(2.4,4.2)	0.02	4.2(3.0, 5.4)	3.1(2.4,4.0)	<0.01	0.43	0.28
Med HDL particle concentration	8.8(7.5,10.3)	9.7(8.7,11.8)	<0.01	8.1(7.0, 9.1)	9.2(7.7,11.0)	<0.01	0.09	<0.01
Lrg HDL particle concentration	3.2(2.1,4.5)	4.2(2.7,6.2)	<0.01	3.0(2.4,4.5)	5.1(3.9,6.7)	<0.01	0.75	<0.01
Tot. HDL particle concentration	18.2(3.0)	20.3(4.3)	<0.01	18.1(4.2)	19.8(4.1)	<0.01	0.30	0.82
HDL cholesterol	53.0(44.0, 64.0)	64.0(55.0, 77.0)	<0.01	53(42, 67)	69(56, 80)	<0.01	0.39	0.55
LDL cholesterol	128.9(35.3)	133.5(41.4)	0.46	107.5(30.1)	102.8(29.3)	0.51	<0.01	<0.01
Non-HDL cholesterol	139(115,174)	142(111, 178)	0.78	125.5(98.0,143.5)	113(96, 139)	0.37	<0.01	<0.01
Total cholesterol concentration	192(171, 221)	209(179, 242)	0.07	179(154.5,195.5)	188(165, 213)	0.12	<0.01	<0.01
Triglycerides	82(52,119)	76(52,107)	0.49	68(44, 95.5)	65(44, 84.0)	0.61	0.25	0.22
Systolic blood pressure	112(105, 121)	107(99, 114)	<0.01	116.5(108.5, 128)	115(105, 122)	0.13	0.04	<0.01
Diastolic blood pressure	73(68, 81)	69(65,76)	<0.01	71(63, 77)	64(58, 71)	<0.01	<0.01	<0.01
HbA1c (%)	5.4(5.1, 5.6)	5.4(5.1, 5.6)	0.63	7.6(6.8, 9.0)	8.0(7.4,9.0)	0.32	<0.01	<0.01
Albumin to creatinine ratio	5.5(3.7,10.3)	8.6(5.2,18.7)	<0.01	10.1(7.0, 25.1)	12.3(7.5, 33.3)	0.61	<0.01	<0.01
White blood cell count	5.4(4.7, 6.2)	5.4(4.5, 6.4)	0.62	6.0(5.1,6.7)	6.1(5.0, 7.5)	0.90	<0.01	<0.01
BMI	26.3(24.4,29.0)	25.9(23.4,29.5)	0.51	26.3(23.7 ,30.5)	27.7(23.9,31.9)	0.24	0.64	0.14

	Controls			Type 1 Diabetes			Men (control vs. T1D)	Women (control vs. T1D)
Participant characteristics	**Men (n=97)**	**Women (n=99)**	**p-value**	**Male (n=64)**	**Female (n=79)**	**p-value**	**p-value**	**p-value**
Age(years)	51.1(41.6, 58.5)	50.1(42.9, 56.6)	0.75	49.2(44.9, 54.1)	48.5(45.2, 54.8)	0.86	0.74	0.62
Waist to hip ratio	0.9(0.8, 1.0)	0.8(0.8, 0.9)	<0.01	0.9(.09,1.0)	0.8(0.8, 0.9)	<0.01	0.05	0.93
Pulse	64(56,72)	64(60,70)	0.47	76(67, 83)	74(68,80)	0.32	<0.01	<0.01
Est. glomerular filtration	86.9(78.0, 99.0)	87.2(76.6,102.7)	0.94	86.7(76.1, 102.9)	79.4(66.5, 96.3)	0.02	0.34	<0.01
Est. glucose disposal rate†	-	-	-	7.2(5.6, 8.5)	8.8(6.7, 9.7)	<0.01	-	-
Apolipoprotein A1	143(129, 161)	156(144,179)	<0.01	148(132,168)	170(145, 182)	<0.01	0.33	0.18
Age at T1D onset †	-	-	-	8.4(4.4,11.4)	8.5(5.0, 11.5)	0.60	-	-
Duration of T1D †	-	-	-	40.7(35.8, 46.1)	40.2(35.6,45.5)	0.85	-	-
Insulin dose per body weight †	-	-	-	0.6(0.5,0.8)	0.5(0.3, 0.6)	<0.01	-	-
Weight in kilograms	81.2(74.9,91.8)	70.7(63.6,81.7)	<0.01	81.9(71.1, 93.4)	74.8(62.2, 83.8)	<0.01	0.95	0.97
Height in centimeters	176(171,183)	165(160, 170)	<0.01	175(170, 180)	163(158, 166)	<0.01	0.05	<0.01
Hypertension status			0.03			0.63	0.02	<0.01
No hypertension	84.5% (82)	93.9% (93)		69.4% (45)	73.1% (58)			
Hypertension	15.5% (15)	6.1% (6)		30.6% (19)	26.9% (21)			
Race			0.11			0.25	0.02	0.69
Non-Hispanic White	90.7% (88)	96.0% (95)		100% (64)	97.0% (76)			
African American	8.3% (8)	3.0% (3)		0% (0)	3.0% (3)			
Hispanic White	0% (0)	0% (0)		0% (0)	0% (0)			
Asian	0% (0)	0% (0)		0% (0)	0% (0)			

Participant characteristics	Controls			Type 1 Diabetes			Men (control vs. T1D)	Women (control vs. T1D)
	Men (n=97)	Women (n=99)	p-value	Male (n=64)	Female (n=79)	p-value	p-value	p-value
Ace inhibitor use			0.03			0.10	<0.01	<0.01
No med use	92.8% (90)	99.0% (98)		62.9% (41)	75.6% (60)			
Med use	7.2% (7)	1.0% (1)		37.1% (23)	24.4% (19)			
Hypertension medication use			0.02			0.98	0.03	<0.01
No med use	88.7% (86)	97.0% (96)		75.8% (49)	75.6% (60)			
Med use	11.3% (11)	3.0% (3)		24.2% (15)	24.4% (19)			
LDL lipid medication use			0.22			0.49	<0.01	<0.01
No med use	91.8% (89)	96.0% (95)		59.7% (39)	65.4% (52)			
Med use	8.2% (8)	4.0% (4)		40.3% (25)	34.6% (27)			
Statin medication use			0.11			0.31	<0.01	<0.01
No med use	91.8% (89)	97.0% (96)		59.7% (39)	67.9% (54)			
Med use	8.2% (8)	3.0% (3)		40.3% (25)	32.1% (25)			
Smoking history			0.04			0.63	0.34	0.16
Never smoker	62.9% (61)	67.7% (67)		62.5% (40)	64.6% (51)			
Former smoker	22.7% (22)	28.3% (28)		29.7% (19)	24.1% (19)			
Current smoker	14.4% (14)	4.0% (4)		7.8% (5)	11.4% (9)			

Data are means (SD), median (25^{th}, 75^{th} percentile), or percentage of column(n)

Table 2 Correlates of HDL-P concentration and cholesterol efflux capacity by type 1 diabetes status

	T-HDL-P	XS-HDL-P	S-HDL-P	M-HDL-P	L-HDL-P	J774 CEC	ABCA 1 CEC
Type 1 diabetes							
Age	0.04 (0.62)	0.11 (0.20)	>-0.01 (0.99)	-0.04 (0.60)	0.07 (0.40)	0.12 (0.16)	**0.16 (0.05)**
Age at onset †	-0.09 (0.28)	0.02 (0.82)	>-0.01 (0.97)	-0.08 (0.36)	-0.06 (0.46)	0.07 (0.40)	0.11 (0.20)
Duration †	0.13 (0.13)	0.11 (0.21)	-0.03 (0.76)	0.02 (0.85)	0.14 (0.10)	0.09 (0.30)	0.11 (0.21)
BMI	-0.10 (0.24)	0.08 (0.31)	**0.26 (<0.01)**	0.01 (0.87)	**-0.31 (<0.01)**	-0.05 (0.52)	0.02 (0.79)
WHR	**-0.25 (<0.01)**	**0.29 (<0.01)**	**0.44 (<0.01)**	**-0.21 (0.01)**	**-0.52 (<0.01)**	-0.11 (0.21)	0.11 (0.21)
HbA1c	-0.03 (0.72)	0.11 (0.20)	0.12 (0.14)	-0.06 (0.48)	-0.09 (0.27)	0.11 (0.18)	0.12 (0.15)
SBP	-0.07 (0.42)	**0.17 (0.04)**	**0.17 (0.05)**	-0.12 (0.15)	-0.12 (0.15)	0.12 (0.15)	**0.29 (<0.01)**
DBP	-0.06 (0.47)	0.07 (0.40)	**0.17 (0.05)**	-0.04 (0.60)	**-0.20 (0.02)**	0.04 (0.68)	**0.18 (0.03)**
Pulse	**0.16 (0.06)**	0.03 (0.69)	**0.17 (0.05)**	0.10 (0.21)	<0.01 (0.99)	**0.17 (0.05)**	**0.16 (0.06)**
HDL-C	**0.78 (<0.01)**	**-0.24 (<0.01)**	**-0.38 (<0.01)**	**0.64 (<0.01)**	**0.86 (<0.01)**	**0.53 (<0.01)**	0.06 (0.45)
LDL-C	-0.01 (0.88)	0.11 (0.23)	**0.23 (<0.01)**	-0.04 (0.68)	**-0.18 (0.04)**	**0.22 (0.01)**	**0.30 (<0.01)**
Non-HDL-C	-0.06 (0.51)	**0.21 (0.01)**	**0.37 (<0.01)**	-0.09 (0.27)	**-0.27 (<0.01)**	**0.27 (<0.01)**	**0.40 (<0.01)**
Total cholesterol	**0.36 (<0.01)**	0.05 (0.53)	**0.15 (0.07)**	**0.23 (<0.01)**	**0.18 (0.03)**	**0.56 (<0.01)**	**0.41 (<0.01)**
Triglycerides	-0.03 (0.74)	**0.24 (<0.01)**	**0.52 (<0.01)**	-0.04 (0.66)	**-0.35 (<0.01)**	**0.28 (<0.01)**	**0.47 (<0.01)**
ApoA1	**0.87 (<0.01)**	-0.11 (0.19)	**-0.15 (0.08)**	**0.68 (<0.01)**	**0.75 (<0.01)**	**0.66 (<0.01)**	**0.24 (<0.01)**
ACR	0.11 (0.21)	<0.01 (0.96)	**0.25 (<0.01)**	-0.03 (0.73)	0.02 (0.81)	**0.21 (0.01)**	**0.27 (<0.01)**
eGFR	-0.10 (0.22)	-0.11 (0.21)	**-0.17 (0.04)**	0.03 (0.72)	-0.02 (0.77)	**-0.20 (0.02)**	**-0.27 (<0.01)**
WBC count	-0.04 (0.68)	**0.21 (0.01)**	0.12 (0.17)	0.07 (0.44)	**-0.17 (0.05)**	-0.025 (0.77)	0.06 (0.45)

	T-HDL-P	XS-HDL-P	S-HDL-P	M-HDL-P	L-HDL-P	J774 CEC	ABCA 1 CEC
T-HDL-P	--	**-0.14 (0.08)**	0.01 (0.91)	**0.78 (<0.01)**	**0.76 (<0.01)**	**0.56 (<0.01)**	**0.19 (0.03)**
XS-HDL-P	--	--	**0.25 (<0.01)**	**-0.19 (0.03)**	**-0.27 (<0.01)**	0.01 (0.91)	**0.17 (0.05)**
S-HDL-P	--	--	--	**-0.18 (0.04)**	**-0.42 (<0.01)**	0.10 (0.22)	**0.28 (<0.01)**
M-HDL-P	--	--	--	--	**0.47 (<0.01)**	**0.32 (<0.01)**	0.02 (0.80)
L-HDL-P	--	--	--	--	--	**0.43 (<0.01)**	0.02 (0.81)
J774 CEC	--	--	--	--	--	--	**0.74 (<0.01)**
Controls							
Age	**0.15 (0.04)**	0.10 (0.17)	0.10 (0.15)	0.06 (0.41)	0.06 (0.43)	**0.24 (<0.01)**	**0.30 (<0.01)**
BMI	**-0.21 (<0.01)**	**0.14 (0.05)**	**0.28 (<0.01)**	**-0.14 (0.06)**	**-0.40 (<0.01)**	-0.12 (0.11)	0.05 (0.50)
WHR	**-0.22 (<0.01)**	**0.26 (<0.01)**	**0.45 (<0.01)**	**-0.22 (<0.01)**	**-0.45 (<0.01)**	-0.01 (0.85)	**0.20 (<0.01)**
HbA1c	-0.04 (0.59)	0.05 (0.49)	0.08 (0.28)	0.04 (0.61)	**-0.21 (<0.01)**	0.01 (0.92)	0.12 (0.09)
SBP	-0.03 (0.66)	0.11 (0.12)	**0.15 (0.03)**	-0.08 (0.27)	**-0.13 (0.08)**	0.05 (0.50)	**0.15 (0.05)**
DBP	-0.04 (0.56)	**0.17 (0.02)**	**0.21 (<0.01)**	-0.09 (0.21)	**-0.20 (<0.01)**	0.10 (0.14)	**0.22 (<0.01)**
Pulse	0.07 (0.32)	<0.01 (0.99)	0.10 (0.15)	0.01 (0.85)	-0.04 (0.54)	0.09 (0.22)	0.11 (0.14)
HDL-C	**0.71 (<0.01)**	**-0.18 (0.01)**	**-0.50 (<0.01)**	**0.54 (<0.01)**	**0.82 (<0.01)**	**0.56 (<0.01)**	**0.15 (0.04)**
LDL-C	0.07 (0.39)	**0.19 (0.02)**	**0.17 (0.04)**	-0.05 (0.57)	-0.06 (0.48)	**0.35 (<0.01)**	**0.41 (<0.01)**
Non-HDL-C	-0.04 (0.58)	**0.25 (<0.01)**	**0.36 (0.01)**	-0.10 (0.15)	**-0.25 (<0.01)**	**0.34 (<0.01)**	**0.47 (<0.01)**
Total cholesterol	**0.24 (<0.01)**	**0.18 (0.01)**	**0.21 (<0.01)**	0.09 (0.19)	0.05 (0.45)	**0.57 (<0.01)**	**0.55 (<0.01)**
Triglycerides	**-0.21 (<0.01)**	**0.14 (0.05)**	**0.63 (<0.01)**	**-0.18 (<0.01)**	**-0.55 (<0.01)**	**0.15 (0.03)**	**0.40 (<0.01)**

	T-HDL-P	XS-HDL-P	S-HDL-P	M-HDL-P	L-HDL-P	J774 CEC	ABCA 1 CEC
ApoA1	**0.80 (<0.01)**	0.01 (0.95)	**-0.21 (<0.01)**	**0.55 (<0.01)**	**0.69 (<0.01)**	**0.69 (<0.01)**	**0.34 (<0.01)**
ACR	0.04 (0.55)	**-0.16 (0.03)**	**-0.15 (0.04)**	-0.01 (0.90)	**0.16 (0.02)**	0.05 (0.51)	-0.01 (0.89)
eGFR	**-0.15 (0.03)**	**-0.17 (0.02)**	**-0.15 (0.04)**	-0.07 (0.32)	-0.02 (0.78)	**-0.20 (<0.01)**	**-0.24 (<0.01)**
WBC count	<0.01 (0.99)	0.09 (0.19)	**0.22 (<0.01)**	-0.01 (0.93)	**-0.16 (0.02)**	-0.01 (0.86)	0.02 (0.77)
T-HDL-P	--	-0.07 (0.32)	**-0.19 (<0.01)**	**0.78 (<0.01)**	**0.76 (<0.01)**	**0.55 (<0.01)**	**0.21 (<0.01)**
XS-HDL-P	--	--	**0.29 (<0.01)**	**-0.22 (<0.01)**	**-0.20 (<0.01)**	0.08 (0.29)	0.10 (0.16)
S-HDL-P	--	--	--	**-0.30 (<0.01)**	**-0.60 (<0.01)**	<0.01 (0.97)	**0.17 (0.02)**
M-HDL-P	--	--	--	--	**0.53 (<0.01)**	**0.30 (<0.01)**	0.07 (0.34)
L-HDL-P	--	--	--	--	--	**0.42 (<0.01)**	0.04 (0.60)
J774 CEC	--	--	--	--	--	--	**0.75 (<0.01)**

* Data are correlation coefficients (p-value)

Table 3 Independent correlates of HDL particle concentration and cholesterol efflux capacity

	T-HDL-P	XS-HDL-P	S-HDL-P	M-HDL-P	L-HDL-P	J774	ABCA1
Type 1 diabetes	-0.74 (-1.2, -0.29) (<0.01)	-0.009 (-0.08, 0.06) (0.80)	0.09 (-0.19, 0.37) (0.52)	-1.06 (-1.46, -0.67) (<0.01)	0.39 (0.10, 0.68) (<0.01)	0.26 (0.22, 0.30) (<0.01)	0.95 (0.79, 1.10) (<0.01)
Females	0.21 (-0.26, 0.68) (0.38)	-0.19 (-0.26, -0.13) (<0.01)	-0.37 (-0.65, -0.10) (<0.01)	0.45 (0.05, 0.85) (0.03)	0.26 (-0.03, 0.55) (0.07)	-0.03 (-0.07, 0.006) (0.10)	-0.15 (-0.30, -0.01) (0.04)
HDLc	0.024 (0.01, 0.05) (0.05)	-0.005 (-0.009, -0.001) (<0.01)	-0.05 (-0.07, -0.04) (<0.01)	-	0.08 (0.06, 0.09) (<0.01)	0.004 (0.002, 0.006) (<0.01)	-
Non-HDL	-	0.001 (0.0003, 0.002) (<0.01)	0.004 (0.0001, 0.01) (0.05)	-	-	0.001 (0.0008, 0.002) (<0.01)	0.005 (0.003, 0.007) (<0.01)
Triglycerides	-	-	0.009 (0.007, 0.01) (<0.01)	-0.005 (-0.008, -0.002 (<0.01)	-0.004 (-0.006, -0.001) (<0.01)	0.001 (0.0008, 0.001) (<0.01)	0.004 (0.003, 0.005) (<0.01)
Albumin to creatinine ratio	-	-	-	-	-	-	0.001 (0.0001, 0.002) (0.04)
White blood cell count	-	0.03 (0.008, 0.05) (<0.01)	-	-	-0.10 (-0.19, -0.01) (0.02)	-	-
BMI	-	-	0.02 (-0.0004, 0.03) (0.06)	-	-0.02 (-0.04, -0.0007) (0.04)	-	-
Estimated glomerular filtration rate	-	-0.003 (-0.005, -0.001) (<0.01)	-0.01 (-0.02, -0.002) (0.01)	-	-	-0.002 (-0.003, -0.0009) (<0.01)	-0.01 (-0.01, -0.004) (<0.01)
Apolipoprotein A1	0.11 (0.09, 0.12) (<0.01)	0.003 (0.0007, 0.005) (0.01)	0.03 (0.02, 0.04) (<0.01)	0.05 (0.04, 0.06) (<0.01)	0.01 (0.003, 0.02) (0.01)	0.005 (0.003, 0.006) (<0.01)	0.01 (0.008, 0.01) (<0.01)
Hypertension	-	-	-	-	-	0.07 (0.02, 0.11) (<0.01)	0.34 (0.15, 0.52) (<0.01)
Never smoker	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Former smoker	-	-	0.24	-0.02	-	-	-

			(-0.06, 0.54) (0.12)	(-0.47, 0.43) (0.94)			
Current smoker	-	-	0.65 (0.20, 1.10) (<0.01)	-0.71 (-1.38, -0.04) (0.04)	-	-	-
R^2	0.74	0.21	0.52	0.46	0.71	0.74	0.61

Data are β (95% Confidence interval) (p-value)

5.0 Discussion

In this study of middle-aged adults, having a diagnosis of childhood-onset type 1 diabetes was independently associated with significantly lower concentrations of M-HDL-P but slightly higher concentrations of L-HDL-P compared with normal glucose tolerance, resulting in type 1 diabetes being independently associated with lower overall T-HDL-P concentrations. Furthermore, type 1 diabetes was associated with significantly higher total and ABCA1-specific CEC after multivariable adjustments, including for traditional lipid levels. Our results therefore suggest greater CEC per HDL-P in type 1 diabetes compared with individuals with normal glucose tolerance, perhaps as a compensatory mechanism in this high cardiovascular risk population. These associations were not modified by sex.

The role of HDL-C as a determinant of cardiovascular disease risk has long been contemplated in type 1 diabetes. Its concentrations, which numerous epidemiologic studies have shown to be strongly, negatively, associated with the development of cardiovascular disease,[78,79] are markedly increased in type 1 diabetes when glucose is closer to physiologic levels.[80–82] Yet, patients with type 1 diabetes are at a greatly increased risk of cardiovascular outcomes compared with the general population, particularly at younger ages.[82–85] Moreover, investigators of the Pittsburgh EDC study noted a U-shaped HDL-C – CAD association among women with type 1 diabetes, such that CAD risk increased both at the lowest (<50 mg/dL) and highest (>80 mg/dL) ends of the HDL-C distribution.[72] Interestingly, recent data from general population studies also suggested either the absence of an association between HDL-C and cardiovascular morbidity and mortality or increased risk with higher HDL-C concentrations.[86,87] These observations, in addition to the failure to show cardiovascular protection in trials of medications known to increase HDL-

C,[88–90] have promoted interest in the ability to assess the structure of the HDL particle and its properties beyond cholesterol concentration. Indeed, HDL's pleiotropic activities include antioxidant, anti-inflammatory, and antithrombotic effects as well as reverse cholesterol function and all these properties are thought to contribute to its characterization as an atheroprotective molecule. Assays have recently been developed to quantify HDL particle number and mean particle size, and addressing each of the above-mentioned activities of HDL. However, to date, there is no single assay to measure HDL function. Moreover, there are currently no standards for the assessment of these novel HDL metrics, making comparison across studies difficult.

Our findings relating to HDL particle concentrations quantified using ion mobility by type 1 diabetes status are similar to previously published results assessing HDL particle concentration by Nuclear Magnetic Resonance Spectroscopy (NMR) in a smaller sample of adults in their 30s. Ahmed et al.[91] reported lower total, medium and small HDL particle concentrations and higher large HDL particle concentrations in individuals with type 1 diabetes compared with controls. Interestingly, a small study among youth also reported lower medium HDL particle concentration by NMR, although small HDL particle concentration was higher among youth with type 1 diabetes compared with controls, and total HDL particle concentrations did not differ between the two groups.[92] However, as sex hormones affect the concentrations of lipoproteins [93] and as pubertal onset may be delayed in children with type 1 diabetes,[94] these results are difficult to interpret. Nevertheless, it appears that there is a shift toward larger HDL particles and lower medium HDL particle concentration in type 1 diabetes that is manifest in young adulthood and continues to middle/older age.

Interestingly, the observed differences in medium and large HDL particle concentration in the present study were more pronounced among women, although no significant effect

modification was observed in multivariable models. In the study by Ahmed et al.,[91] univariate differences by type 1 diabetes status in total and small HDL particle concentrations were significant only among women, although results for medium and large HDL particle concentrations did not differ by sex. Unfortunately, sex-specific differences by type 1 diabetes status were not reported by Gourgari et al.[92] Whether the sex differences observed in the present study may explain the increased relative risk of cardiovascular outcomes in women with type 1 diabetes cannot be determined from the present results but should be pursued in future studies.

Despite the lower concentrations of total HDL particles among study participants with type 1 diabetes, both total macrophage and ABCA1-specific CEC were higher compared with controls. These associations, which were maintained after multivariable adjustments, were not modified by sex. However, in multivariable models, ABCA1-specific CEC was lower in women compared with men regardless diabetes status. Work published by Du et al.[95] suggesting that smaller forms of HDL are the main promoters of sterol efflux from macrophages by the ABCA1 pathway, support our findings, as women appear to have lower concentrations of XS- and S-HDL-P in this study. However, the implications of these findings regarding sex differences in the risk for cardiovascular complications in type 1 diabetes are unclear.

Total CEC was greater in type 1 diabetes compared with non-diabetic controls also in the study by Ahmed et al.,[91] although the difference for ABCA1-specific CEC was less marked and rendered non-significant in multivariable models. In contrast, univariately, CEC was lower among youth with type 1 diabetes compared with healthy control youth in the study by Gourgari et al.,[92] although no multivariable analyses were conducted. Neither effect modification nor analyses stratified by sex was conducted in these smaller studies.

Strengths of our study include a large, well-characterized, cohort of middle-aged adults with type 1 diabetes and non-diabetic controls of a similar age and sex distribution. Our assessments comprised well validated assays of HDL particle concentration and CEC. Moreover, our investigation of differences in HDL particle concentration and CEC by type 1 diabetes status evaluated the presence of effect modification by sex. Limitations include the cross-sectional study design and the racially homogenous cohort; only 4% of study participants were not non-Hispanic white, limiting the generalizability of the study results. However, the distribution of race/ethnicity in this study depended on that of type 1 diabetes patients in Allegheny County, PA in the period they were diagnosed with type 1 diabetes (1950-80). In addition, our population includes individuals who have survived with type 1 diabetes for a median of 40 years and, therefore, our findings may not apply to all those diagnosed with this chronic disease.

In conclusion, our results demonstrate differences in HDL particle concentration and CEC by sex and type 1 diabetes status. Whether these differences exist at the diagnosis of type 1 diabetes or are a result of metabolic abnormalities in type 1 diabetes is not clear at present. However, future research studies should address the public health implications of these findings in terms of subsequent development of cardiovascular complications.

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